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A Natural Host and Diversity of *Pepper Vein Yellows Virus* in Japan

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Abstract

Pepper vein yellows virus (PeVYV, *Luteovirdae*; *Polerovirus*) infects *Capsicum* plants, whose bell pepper fruits (*Capsicum annuum*) are important crops in Japan. Reductions in the yield and quality of bell pepper fruits caused by PeVYV are agricultural problems in Okinawa Prefecture, Japan. The prevalence of PeVYV deposits in the annual disposition of harvested bell pepper plants suggests the possible existence of a reservoir for the virus. Here, we examined bird pepper plants (*Capsicum frutescens*) that were grown wild on five islands in Okinawa Prefecture, as well as cultivated peppers (*C. annuum*, *Capsicum baccatum* and *C. frutescens*) for the presence of PeVYV by reverse transcription polymerase reactions using specific primers. Overall, PeVYV was widely detected in bird peppers on the three islands (Okinawa, Miyako and Yonaguni) where the bell pepper disease occurred, but not detected in the plants from two islands (Ishigaki and Minami-Daito) with no disease. Laboratory experiments demonstrated that an Okinawa-derived strain of cotton aphid *Aphis gossypii* transmitted PeVYV from viruliferous wild bird peppers to the bell peppers, which may suggest an annual supply of PeVYV from wild bird peppers to the bell peppers via aphids in Okinawa Prefecture. Molecular phylogenetic analyses based on the nucleotide sequences of seven distinct genomic regions suggested that PeVYV detected from the bell peppers and bird peppers are indistinguishable, supporting the view that PeVYV moves frequently between these host plants.

Discipline: Insect pests / Plant disease
Additional key words: Aphid, *Aphis gossypii*, PeVYV, transmission

Introduction

Pepper crops (*Capsicum* spp.) have a wide geographic distribution and are economically important worldwide. Pepper plants are frequently infected by both non-persistent and persistent aphid-transmitted viruses. It is difficult to control viruses transmitted by insect vectors given the small size of vector insects and the movement of insects. In Japan, bell pepper fruits (*Capsicum annuum*) are important crops, and *pepper vein yellows virus* (PeVYV; family *Luteoviridae*, genus *Polerovirus*), which infects bell pepper plants (Fig. 1), poses a significant problem. Bell pepper diseases have been observed every year in Okinawa Prefecture since 1981, and PeVYV is transmitted by *Aphis gossypii* (Yonaha et al. 1995). In Okinawa Prefecture, bell pepper seedlings are generally planted in plastic greenhouses from August to September, with the bell pepper fruits being harvested from October to June. The harvested bell pepper plants are discarded. However, bell pepper diseases have occurred every year and the source of infection is not always

Fig. 1. Symptoms of pepper vein yellows virus: yellowed veins of the bell pepper leaves.

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known. Wild bird peppers (*C. frutescens*) grow naturally throughout the year in Okinawa Prefecture, and *A. gossypii* survives on the plants. PeVYV infects *Capsicum* plants and *A. gossypii* attacks a variety of plants. Therefore, we hypothesized that the source of infection in bell pepper disease was PeVYV initially in wild viruliferous bird peppers and then transmitted by *A. gossypii* from the viruliferous bird peppers to healthy bell peppers. Both the detection of PeVYV in wild bird peppers and its transmission from wild bird peppers to bell peppers by *A. gossypii* were confirmed in the present study.

After the RNA complete genome of PeVYV was sequenced in 2011 (Murakami et al. 2011), many other sequences of PeVYV have been reported (Alfaro-Fernandez et al. 2014, Buzkan et al. 2013, Knierim et al. 2013, Tan et al. 2015, Villanueva et al. 2013, Zang et al. 2015). In the evolution of RNA virus, recombinations are general phenomena, and RNA recombination is considered a major driving force in virus variability. Traces of recombination events can be detected in poleroviruses, and there are traces of recombination in the intergenic region (IR) and some open reading frames (ORFs) of poleroviruses (Elsayed et al. 2014, Pagan et al. 2010). PeVYV also shows recombination breakpoints in ORF coding coat protein (ORF3) or a readthrough domain (RTD: ORF5) (Dombrovsky et al. 2013). To examine the nucleotide variations of PeVYV in Japan, we conducted phylogenetic analysis to compare the partial RNA dependent RNA polymerase (ORF2) sequences, ORF3 sequences, movement protein (ORF4) sequences, ORF5 sequences, and IR sequences of PeVYV sampled from Okinawa Prefecture with the sequences of PeVYV from other countries.

Materials and methods

Aphid transmission: Transmissions by *A. gossypii* were performed as described by Yonaha et al. (1995). The Oki strain of *A. gossypii* was collected from bell pepper plants in Okinawa Prefecture; the Shizu strain was collected from *Cucumis sativus* plants in Shizuoka Prefecture (Table 1). The H11 and H13 strains were collected from *Solanum tuberosum* plants in Hokkaido, the GSM strain was collected from *S. melongena* plants in Gifu Prefecture, and the Iba strain was collected from *Lycopersicon esculentum* plants in Ibaraki Prefecture. These aphids were reared on the seedlings of tic beans (Murai 1991) and fed on viruliferous pepper plants for five days to intake PeVYV. Five adult aphids were transmitted onto 10 tested plants for seven days during inoculation testing, and the infested plants were then sprayed with an insecticide (Venica X spray, Sumitomo Chemical Garden Products, Tokyo, Japan) to remove the aphids. The plants were then maintained in a greenhouse at 25°C. We judged PeVYV transmission to be successful when symptoms of PeVYV appeared on plants and reverse transcription polymerase chain reaction (RT-PCR) amplicons of PeVYV were detected in the infected plants.

RNA Extraction from plants: Seventy micrograms of plants were ground with 50 mg of sea sand in 1 ml of RNAiso (Takara, Shiga, Japan). Two hundred microliters of chloroform were added to the solution, and then the aqueous phase was collected. RNAs were precipitated by the addition of 400 µl of isopropanol to the solution and washed with 500 µl of 70% ethanol. The precipitated RNAs were dissolved in 50 µl of RNase-free water to use for RT and PCR.

Extraction of RNAs from aphids: One adult aphid was

Table 1. Survival of each *Aphis gossypii* strain on plants and transmission of PeVYV by the *A. gossypii* strains to plants.

	Aphis gossypii strain											
	Oki		Shizu		H11		H13		GSM		Iba	
Plants	Sur[1)]	Trans[2)]	Sur	Trans	Sur	Trans	Sur	Trans	Sur	Trans	Sur	Trans
Brassica rapa	+	−	+	−	+	−	+	−	+	−	+	−
Capsicum annum	+	+	+	+	+	+	+	+	+	+	+	+
Capsicum frutescenes	+	+	−	−	−	−	−	−	−	−	−	−
Cucumis sativus	−	−	+	−	+	−	+	−	+	−	−	−
Lycopersicon esculentum	+	−	+	−	+	−	+	−	+	−	+	−
Nicotiana tabacum	−	−	−	−	−	−	−	−	−	−	−	−
Raphanus sativus	+	−	+	−	+	−	+	−	+	−	+	−
Vicia faba	+	−	+	−	+	−	+	−	+	−	+	−

1) +: Aphis had survived on the plant at 14 days after inoculation; −: aphids did not survive.
2) +: PeVYV was transmitted from viruliferous bell peppers; −: PeVYV was not transmitted.

ground into 100 µl of RNAiso, followed by 20 µl of chloroform being added to the solution. The aqueous phase was then collected. RNAs were precipitated by the addition of 40 µl of isopropanol and washed with 50 µl of 70% ethanol. The precipitated RNAs were dissolved in 5 µl of RNase-free water.

RT-PCR for detection of PeVYV: Viral cDNAs were amplified from 1 µl of RNA solution in 20 µl of SuperScript™ One-Step RT-PCR with Platinum Taq (Invitrogen, Tokyo, Japan) reaction solution using specific primer sets (PeVYV-f / PeVYV-r, Table 2) to detect PeVYV. The specific primers were designed in RTD to detect only PeVYV because the RTD of PeVYV has unique sequences. Five microliters of the reacted solution were loaded onto 1% agarose gel, and electrophoresis was carried out at 100 V for 30 min. To determine the quality of the extracted RNAs, RT-PCR amplicons of nicotinamide adenine dinucleotide hydride (NADH) dehydrogenase were detected using nad5-f primer and nad5-r primer (Menzl et al. 2002) for plants, and elongation factor alpha 1 was examined using EF1-f primer and EF1-r primer for aphids.

Plant sampling: Japanese peppers are *C. annuum*, *C. baccatum* and *C. frutescens* (Yonamine et al. 2013). From 2012 to 2014, we collected wild bird peppers (*C. frutescens*) from nine locations on five islands (Ishigaki, Minami-Daito, Miyako, Okinawa and Yonaguni) in Okinawa Prefecture (Fig. 2 and Table 3). Okinawa Island is the largest of the five islands, and had five sampling locations. Three cultivated peppers (*C. annuum*, *C. baccatum* and *C. frutescens*) were collected from cultivated fields at the Okinawa Prefectural Agricultural Research Center in Itoman on Okinawa Island. Nine plant samples were obtained from each location, and the detection rate of PeVYV at each location was calculated based on the sampled plants.

Table 2. List of selected primers used for the amplification of the *pepper vein yellows virus* (PeVYV).

Name	Position(bp)	5'-3' sequence	Target
PeVYV-f	5085 - 5105	GAGAAAGACAACTGTTAAAACTCCA	PeVYV
PeVYV-r	5688 - 5714	TCGTGAGTTTAGGTACTATTCCTTCCT	PeVYV
nad5-f		GATGCTTCTTGGGGCTTCTTGTTT	*Capsicum spp.*
nad5-r		CTCCAGTCACCAACATTGGCATAA	*Capsicum spp.*
EF1-f		CGTTCCATCCAGAGATGGGAACAAAG	*Aphis gossypii*
EF1-r		GATCGTTGGTGTGAACAAGATGGACTC	*Aphis gossypii*
pevyv 648-671f	648 - 671	TCGTTTGTCTCGCGTTGCTTTTCG	PeVYV
pevyv 2017-1994r	1994 - 2017	ATCGTTGGAACCCTGGGATCTCTT	PeVYV
pevyv 1822-1845f	1822 - 1845	GGAGAAGCTAGTGGAGAGAATCGA	PeVYV
pevyv 2754-2735r	2735 - 2754	CTCCAGCACTTGCTCATCCG	PeVYV
pevyv 2524-2548f	2524 - 2548	CTTGTTCAAGCTGGTCTCTGTGATC	PeVYV
pevyv 3118-3097r	3097 - 3118	CCATCGCCATGGCCCAGTCAGC	PeVYV
pevyv 3059-3083f	3059 - 3083	ATTCAAGGATCCGAGTTATGGCCGC	PeVYV
pevyv 3687-3654r	3654 - 3687	GTGTTACGTGATCCACCATTTCCATTATTATTTC	PeVYV
pevyv 3550-3570f	3550 - 3570	TTCTTATCCGCAATCCCAATT	PeVYV
pevyv 4268-4244r	4244 - 4268	GGGTACTACCTTCTACCTATTTCGG	PeVYV
pevyv 4186-4120f	4186 - 4120	GACGAAGAACGTTGCCGCCGGTTTC	PeVYV
pevyv 4795-4771r	4771 - 4795	AGGAAGCGAACCAATCAACTTCAACT	PeVYV
pevyv 4691-4721f	4691 - 4721	AATTATCAGACGAATATCACCTTTGTAGCGC	PeVYV
pevyv 5277-5253r	5253 - 5277	TGGTCTTGTGACTCACTGAGCTCGA	PeVYV
pevyv 5019-5116f	5019 - 5116	GACAACTCGTTAAAACTCCATCTCCG	PeVYV
pevyv 5694-5671r	5671 - 5694	CCTTCCTCAACTGTCCTCTTAGAC	PeVYV
pevyv 5602-5625f	5602 - 5625	GTCTCTGAGACGGGGTTCTGAAGC	PeVYV
pevyv 6186-6162r	6162 - 6186	TACTTCCACACAGATCTGTCTGCGGG	PeVYV

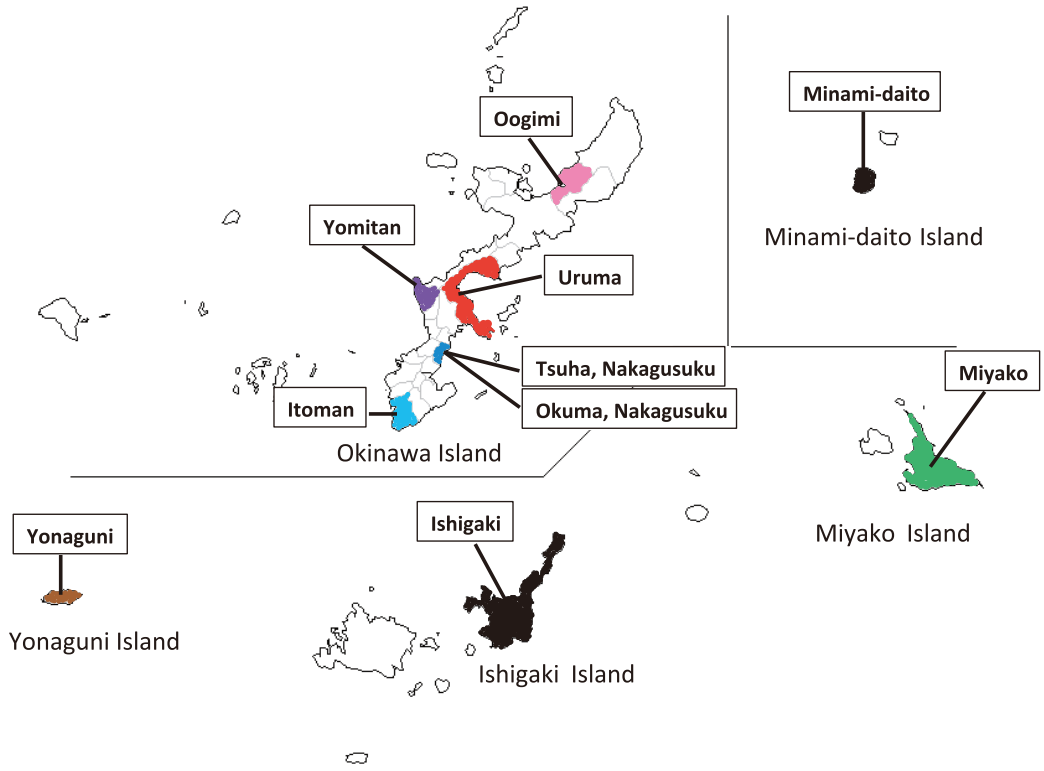

Fig. 2. Sampling locations.
Colors indicate locations where bell pepper diseases have been found: pink: Oogimi village; red: Uruma; blue: Nakagusuku village including Tsuha and Okuma; light blue: Itoman; purple: Yomitan village; brown: Yonaguni Island; and green: Miyako Island. Black (Ishigaki Island and Minami-Daito Island) indicates locations where the diseases have not been reported.

Table 3. Details of PeVYV detection from plants.

Collection date	Provenance (scientific name)	Condition	Locality	Island	%[1)]	Disease[2)]
2012/3/7	Bird pepper (*Capsicum frutescens*)	Wild	Oogimi	Okinawa	100	+
2012/3/16	Bird pepper (*C. frutescens*)	Wild	Miyako	Miyako	44.4	+
2012/3/19	Bird pepper (*C. frutescens*)	Wild	Uruma	Okinawa	100	+
2012/3/21	Bird pepper (*C. frutescens*)	Wild	Tsuha, Nakagusuku	Okinawa	55.6	+
2012/3/21	Bird pepper (*C. annuum*)	Cultivated	Okuma, Nakagusuku	Okinawa	44.4	+
2012/8/14	Bird pepper (*C. frutescens*)	Wild	Yomitan	Okinawa	55.6	+
2012/11/21	Bird pepper (*C. frutescens*)	Wild	Yonaguni	Yonaguni	100	+
2012/11/21	Bird pepper (C. frutescens)	Wild	Ishigaki	Ishigaki	0	−
2013/1/22	Bird pepper (*C. frutescens*)	Wild	Minami-Daito	Minami-Daito	0	−
2014/7/4	Bird pepper (*C. annuum*)	Cultivated	Itoman	Okinawa	100	+
2014/7/4	Bird pepper (*C. baccatum*)	Cultivated	Itoman	Okinawa	100	+
2014/7/4	Bird pepper (*C. frutescens*)	Cultivated	Itoman	Okinawa	100	+

[1)] rates of PeVYV detection, [2)] +: bell pepper diseases caused by PeVYV have been repoted from this island; −: no bell pepper disease reported.

Nine bell peppers (*C. annuum*) were collected from one greenhouse (Okuma, Nakagusuku village) and five *A. gossypii* were collected from bird peppers at Tsuha, Nakagusuku village on Okinawa Island.

Sequence data analysis: RT-PCR amplicons of PeVYV from the sampled plants were synthesized using primer sets (Table 2, Fig. 3: pevyv648-671f / pevyv2017-1994r, pevyv1822-1845f / pevyv2754-2735r, pevyv2524-2548f / pevyv3118-3097r, pevyv3059-3083f / pevyv3687-3654r, pevyv3550-3570f / pevyv4268-4244r, pevyv4186-4120f / pevyv4795-4771r, pevyv4691-4721f / pevyv5277-5253r, pevyv5019-5116f / pevyv5694-5671r, pevyv5602-5625f / pevyv6186-6162r) based on the PeVYV genome sequence (AB594828), and then sequence reaction was performed using a BigDye^{R} Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan). Sequencing analyses of RT-PCR amplicons were conducted using an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems), with sequence data of the amplicons being assembled using ATGC ver. 4.2 (Genetyx, Tokyo, Japan).

Phylogenetic analysis: The nucleotide sequences of ORF2, ORF3, ORF4, ORF5 and IR of PeVYV were retrieved from GenBank, with the retrieved sequences and Japanese sequences being aligned using ClustalW (Thompson et al. 1994). The phylogenetic relationships among PeVYV isolates were determined using MEGA version 6.06 (Tamura et al. 2013) and clustered by the maximum likelihood (ML) algorithm with 1000 replications. The sequences positioned at 2970-3422 bp, 3632-4252 bp, 3663-4133 bp, 4253-5842 bp and 3481-3631 bp of PeVYV (AB594828) were used for the analyses of ORF2, ORF3, ORF4, ORF5 and IR, respectively.

Results

Aphid transmission

The Oki strain aphid died on *Cucumis sativus* and *Nicotiana tabacum*, and only the Oki strain survived on *C. frutescens* (Table 1). All strains transmitted PeVYV from viruliferous bell peppers to healthy bell peppers, but only the Oki strain transmitted PeVYV from bell peppers to bird peppers (*C. frutescens*). Moreover, only strains from Okinawa Prefecture transmitted PeVYV from viruliferous bell peppers to bird peppers and from viruliferous bird peppers to bell peppers (Table 4). Infected bird peppers showed symptoms similar to those of bell peppers infected with PeVYV. When the presence of PeVYV in plants showing these symptoms was checked by RT-PCR, RT-PCR amplicons of PeVYV were detected at approximately 634 bp (Fig. 4a). RT-PCR amplicons were detected in aphids that transmitted PeVYV from viruliferous plants to healthy plants.

Detection of PeVYV from plants in fields

RNAs were extracted from bird peppers sampled from nine locations, and the percentages of PeVYV detection ranged from 0 to 100% (Table 3). RT-PCR amplicons of NADH dehydrogenase were detected in all samples (181 bp, Fig. 4b), but RT-PCR amplicons of PeVYV were not detected in the samples from Ishigaki Island or Minami-Daito Island, where no bell pepper disease has

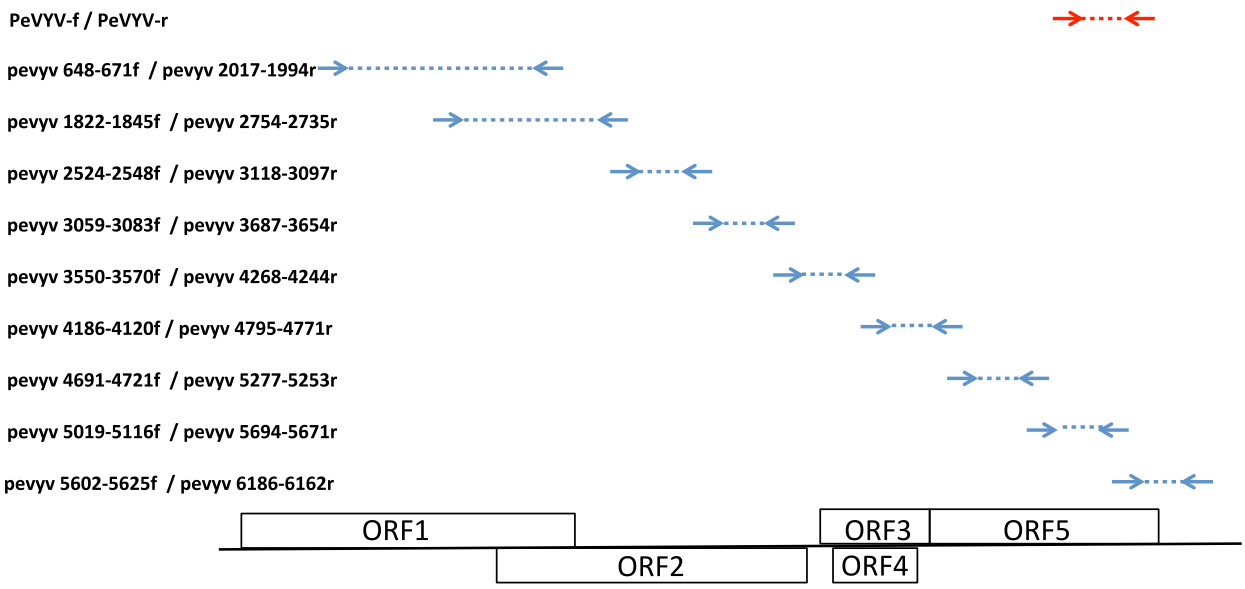

Fig. 3. Genetic map of a part of pepper vein yellows virus and used primers.
Upper: pairs of used primers (left) and position of primers on PeVYV genome (right). Lower: scheme of PeVYV genome. Squares represent the open reading frame.

Table 4. Transmission of PeVYV by *Aphis gossypii* strain.

Strain of *A. gossypii* (Prefecture)	Month and year tested	Bell pepper → Bird pepper	Bird pepper → Bell pepper
Oki	August 2012	9 / 10	9 / 10
(Okinawa)	July 2013	10 / 10	10 / 10
	August 2014	10 / 10	10 / 10
Shizu	August 2012	0 / 10	0 / 10
(Shizuoka)	July 2013	0 / 10	0 / 10
	August 2014	0 / 10	0 / 10
H11	August 2012	0 / 10	0 / 10
(Hokkaido)	July 2013	0 / 10	0 / 10
	August 2014	0 / 10	0 / 10
H13	August 2012	0 / 10	0 / 10
(Hokkaido)	July 2013	0 / 10	0 / 10
	August 2014	0 / 10	0 / 10
GSM	August 2012	0 / 10	0 / 10
(Gifu)	July 2013	0 / 10	0 / 10
	August 2014	0 / 10	0 / 10
Iba	August 2012	0 / 10	0 / 10
(Ibaraki)	July 2013	0 / 10	0 / 10
	August 2014	0 / 10	0 / 10
Tsuha Nakagusu	August 2012	10 / 10	10 / 10
(Okinawa)	July 2013	10 / 10	10 / 10
	August 2014	10 / 10	10 / 10

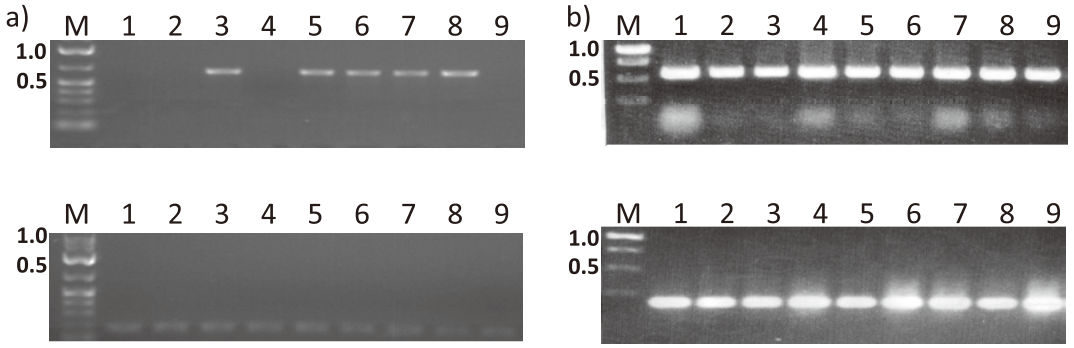

Fig. 4. Detection of RT-PCR amplicons of PeVYV from bird peppers (a) and from *Aphis gossypii* (b).
M: molecular marker from Nippon gene (a) and Daiichi Pure Chemicals (b) 1-9: PeVYV detection in samples (upper images) and RNA quality check of the samples (lower images).

been reported.

At sampling, *A. gossypii* existed on bird pepper from the Tsuha, Nakagusuku location, and the aphids (Tsuha Nakagusuku strain in Table 4) increased on healthy bell peppers. The increased aphids transmitted PeVYV from viruliferous bird peppers to healthy bell peppers. Amplicons of PeVYV RT-PCR were detected in the aphids.

Phylogenetic relationships of PeVYV in Japan

In the 195 RT-PCR amplicons detected, 26 sequences

from seven locations were analyzed. Fourteen sequences were found to have ORFs from ORF1 to ORF5, three sequences had ORFs from ORF2 to ORF5, eight sequences had ORFs from ORF3 to ORF5 (Table 5), and one sequence had ORF1, ORF2 and IR. The phylogenetic relationships among PeVYV isolates were determined by an ML algorithm based on evaluation using the best fit substitution model for ORF2 (Fig. 5a), and the Akaike information criterion value with the TN93+G+I model was the lowest among the 24 models tested. For ORF3 (Fig. 5b), ORF4 (Fig. 5c) and ORF5 (Fig. 5d), AICcs were lowest for the GTR+G+I model, TN93+I model, and GTR+G model, respectively. In these analyses, the bootstrap values were low and Japanese sequences did not differ from the sequences from other countries. Moreover, we found no significant differences among the ORFs of Japanese strains. IR sequences from Japan were then compared with those from other countries to compare accumulations of RNA variations in the IR with accumulations in ORFs. For the IR (Fig. 5e), AICcs were lowest for the T92+G+I model and the bootstrap values were low. There were no significant differences among the IR sequences of PeVYV.

Discussion

It has been reported that PeVYV was detected infecting a nightshade species in India (Knierim et al. 2013), but further data are required as overwintering reservoirs of

Table 5. Sequenced region of PeVYV from Japan.

Accession No.	Sequenced region	Location
LC126030	ORF1(par) - ORF2 - IR - ORF3 - ORF4 - ORF5	Oogimi
LC126031	ORF1(par) - ORF2 - IR - ORF3 - ORF4 - ORF5	Oogimi
LC126032	ORF3 - ORF4 - ORF5	Oogimi
LC126033	ORF1(par) - ORF2 - IR - ORF3 - ORF4 - ORF5	Oogimi
LC126034	ORF3 - ORF4 - ORF5	Miyako Island
LC126035	ORF1(par) - ORF2 - IR - ORF3 - ORF4 - ORF5	Miyako Island
LC126036	ORF1(par) - ORF2 - IR - ORF3 - ORF4 - ORF5	Uruma
LC126037	ORF3 - ORF4 - ORF5	Uruma
LC126038	ORF3 - ORF4 - ORF5	Uruma
LC126039	ORF1(par) - ORF2 - IR - ORF3 - ORF4 - ORF5	Uruma
LC126040	ORF1(par) - ORF2 - IR - ORF3 - ORF4 - ORF5	Uruma
LC126041	ORF1(par) - ORF2 - IR - ORF3 - ORF4 - ORF5	Uruma
LC126042	ORF1(par) - ORF2 - IR - ORF3 - ORF4 - ORF5	Yomitan
LC126043	ORF1(par) - ORF2(par) - IR - ORF3 - ORF4 - ORF5	Yomitan
LC126044	ORF1(par) - ORF2(par) - IR - ORF3 - ORF4 - ORF5	Yomitan
LC126045	ORF1(par) - ORF2 - IR	Nakagusuku
LC126046	ORF2(par) - IR - ORF3 - ORF4 - ORF5	Nakagusuku
LC126047	ORF2(par) - IR - ORF3 - ORF4 - ORF5	Nakagusuku
LC126048	ORF1(par) - ORF2(par) - IR - ORF3 - ORF4 - ORF5	Nakagusuku
LC126049	ORF1(par) - ORF2(par) - IR - ORF3 - ORF4 - ORF5	Nakagusuku
LC126050	ORF2(par) - IR - ORF3 - ORF4 - ORF5	Nakagusuku
LC126051	ORF3 - ORF4 - ORF5	Yonaguni Island
LC126052	ORF3 - ORF4 - ORF5	Yonaguni Island
LC126053	ORF3 - ORF4 - ORF5	Itoman
LC126054	ORF1(par) - ORF2 - IR - ORF3 - ORF4 - ORF5	Itoman
LC126055	ORF3 - ORF4 - ORF5	Itoman

(par): partial sequence.

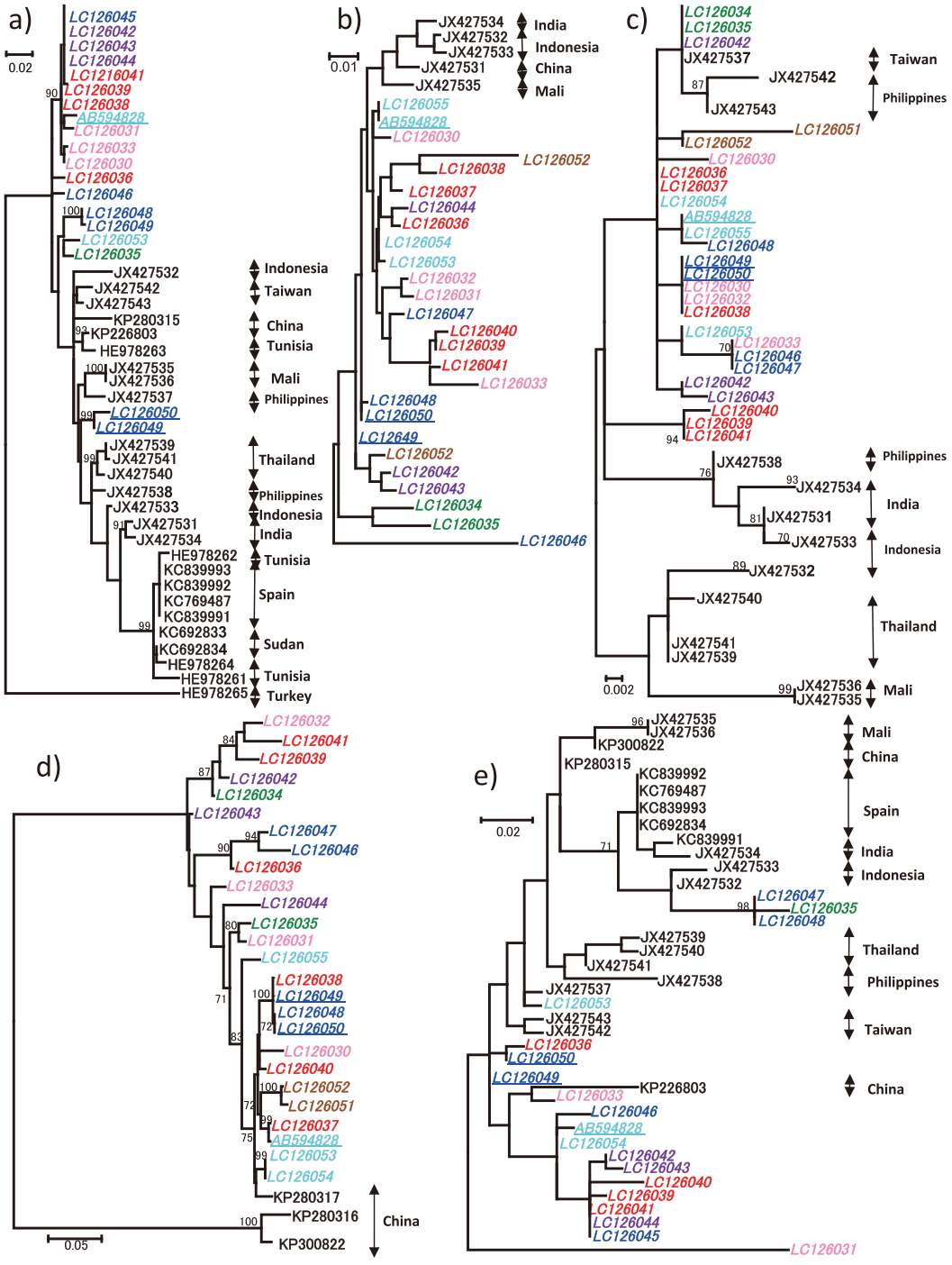

Fig. 5. Phylogenetic analysis showing the predicted relationships between pepper vein yellows virus (PeVYV) isolates based on nucleotide sequences: (a) partial ORF2 (452 nt), (b) partial ORF3 (621 nt), (c) partial ORF4 (471 nt), (d) partial ORF5 (1589 nt), and (e) intergenic region (199 nt).

Horizontal lines are in proportion to the number of nucleotide substitutions between branch nodes. Only bootstrap values above 70% are shown. The scale shows substitutions per site. Italicized Accession Nos. show Japanese PeVYV, and AB594828 is a sequence of Japanese PeVYV from Itoman in Okinawa Prefecture. Underlined Accession Nos. show sequences of PeVYV from bell pepper, and colored Accession Nos. show Japanese sequences: pink, Oogimi village; red, Uruma; blue, Nakagusuku village; light blue, Itoman; purple, Yomitan village; brown, Yonaguni Island; and green, Miyako Island. Black Accession Nos. show sequences from other countries: China, KP226803, KP280315, KP280316, KP280317, KP300822; India, JX427531, JX427534; Indonesia, JX427532, JX427533; Mali, JX427535, JX427536; Philippines, JX427537, JX427538; Spain, KC769487, KC839991, KC839992, KC839993; Sudan, KC692833, KC692834; Taiwan, JX427542, JX427543; Thailand, JX427539, JX427540, JX427541; Tunisia, HE978261, HE978262, HE978263, HE978264; and Turkey, HE978265.

the disease are currently unknown (Kenyon et al. 2014). Although harvested bell pepper plants are discarded every year in Japan, the bell pepper diseases caused by PeVYV nevertheless occur every year. On the other hand, wild bird peppers with yellow veins have been observed throughout Okinawa Prefecture. At Nakagusuku village in Okinawa Prefecture, the Tsuha sampling location is near the Okuma location. The present study found that there were viruliferous bird peppers in Tsuha and bell peppers infected with PeVYV in Okuma. Moreover, PeVYV was not detected in wild bird peppers from the islands where no bell pepper diseases have been reported. With respect to *A. gossypii*, only strains from Okinawa Prefecture transmitted PeVYV from viruliferous bird peppers to bell peppers (Table 4) and survived on both types of peppers. *Aphis gossypii* is highly polyphagous and adaptable to various plants. Because bird peppers grow naturally only in Japan's Okinawa Prefecture, only *A. gossypii* in Okinawa Prefecture may adapt the plants. These present results indicate that bell pepper diseases are caused by PeVYV transmission by *A. gossypii* from Okinawa Prefecture on viruliferous wild bird peppers due to the unique food habits of *A. gossypii* in Okinawa Prefecture. Therefore, we believe that the exclusion of wild viruliferous bird peppers and the control of *A. gossypii* in Okinawa Prefecture are important for controlling bell pepper diseases, and that preventing the invasion by *A. gossypii* from Okinawa Prefecture and *Capsicum* plants caused by PeVYV may be important to prevent this disease from occurring on other islands in Japan.

In terms of the phylogenetic relationship of PeVYV RNA, the bootstrap values of ORFs were found to be low by the ML method, along with low bootstrap values analyzed by other methods (neighbor-joining method and maximum parsimony method). As ORFs code functional proteins, the varieties of RNA might be affected by functions coding proteins. As the IR sequence has not synthesized proteins, variations of RNA are accumulated in the IR. However, PeVYVs from Japan were found not to differ from PeVYVs from other countries. Thus, we believe that Japanese PeVYVs are similar to those from other countries. PeVYV is a high-prevalence virus in Asia, Africa and Europe. The results of the present study suggest that PeVYV invaded Japan after being distributed over a large area. Moreover, there were no significant differences in the nucleotide sequences of PeVYV between bell peppers and bird peppers. The results might also show that PeVYV moves frequently between bell peppers and bird peppers, and that bird peppers caused by PeVYV are one of reservoir of PeVYV, the pathogen of bell pepper disease.

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References

Alfaro-Fernandez A. et al. (2014) First report of pepper vein yellows virus infecting hot pepper in Sudan. *Plant Dis*. **98**, 1446.

Buzkan N. et al. (2013) High prevalence of poleroviruses in field-grown pepper in Turkey and Tunisia. *Arch. Virol*. **158**, 881-885.

Dombrovsky A. et al. (2013) The complete genomic sequence of pepper *Yellow leaf curl virus* (PYLCV) and its implications for our understanding of evolution dynamics in the genus *Polerovirus. PLOS ONE* 8, e70722.

Elsayed A. I. et al. (2014) Molecular evolutionary history of *Sugarcane yellow leaf virus* based on sequence analysis of RNA-dependent RNA polymerase and putative aphid transmission factor-coding genes. *J. Mol. Evol*. **78**, 349-365.

Kenyon L. et al. (2014) Virus diseases of peppers (*Capsicum spp.*) and their control. *Adv. Virus Res*. **90**, 297-353.

Knierim D. et al. (2013) Analysis of sequences from field samples reveals the presence of the recently described pepper vein yellows virus (genus *Polerovirus*) in six additional countries. *Arch. Virol*. **158**, 1337-1341.

Menzl W. et al. (2002) Detection of four apple viruses by multiplex RT-PCR assays with coamplification of plant mRNA as internal control. *J. Virol. Methods* **99**, 81-92.

Murai, T. (1991) Rearing method for clones of some aphids on the tic bean, *Vicia faba. Bull. Shimane Agric. Exp. Sta*. **25**, 78-82 [In Japanese with English summary].

Murakami R. et al. (2011) The genome sequence of pepper vein yellows virus (family *Luteoviridae*, genus *Polerovirus*). *Arch. Virol*. **156**, 921-923.

Pagan I. et al. (2010) Long-term evolution of the *Luteoviridae*: time scale and mode of virus speciation. *J. Virol*. **84**, 6177-6187.

Tamura K. et al. (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol*. **30**, 2725-2729.

Tan W. P. et al. (2015) The first identification of pepper vein yellows virus in Shandong Province, China. *Plant Dis*. **99**, 1288.

Thompson J. D. et al. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and

weight matrix choice. *Nucl. Acids Res*. **22**, 4673-4680.

Villanueva F. et al. (2013) First report of *Pepper vein yellows virus* infecting sweet pepper in Spain. *Plant Dis*. **97**, 1261.

Yonaha T. et al. (1995) Pepper vein yellows virus, a novel Luteovirus from bell pepper plants in Japan. *Ann. Phytopathol. Soc. Jpn*. **61**, 178-184.

Yonamine K. et al. (2013) Characterization of fruits and identification of bird pepper (*Capsicum spp*.) collected from Okinawa Prefecture in Japan. *Bull. Okinawa Prefectural Agric. Res. Center* **4**, 55-60 [In Japanese].

Zang S. B. et al. (2015) First report of pepper vein yellows virus infecting red pepper in mainland China. *Plant Dis*. **99**, 1190.